

<b>Storage</b>	The master mix is stable when stored at -20°C for 12 months in a constant temperature freezer or at 4°C for up to 3 months. The master mix will survive a minimum of 10 freeze/thaw cycles. Shipped on ice.
<b>Description</b>	A ready to use PCR master mix, without primers and template. Each 25 µl of master mix will give a 50 µl PCR containing 1.25u SuperTherm Gold DNA polymerase mix, 200 µM dNTPs, MgCl <sub>2</sub> , 1 x buffer.
<b>Supplied Reagents</b>	2X PCR Master Mix, supplied as a 5 ml aliquot sufficient for 200 reactions ( 50 µl reaction). 1 x 25mM MgCl <sub>2</sub>
<b>Unit Definition</b>	One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTP's into DNA in 30 minutes at 70°C under the assay conditions below.
<b>Unit Assay Conditions</b>	25 mM TAPS (pH9.3 at 25°C), 50 mM KCl, 2 mM MgCl <sub>2</sub> , 1 mM B-mercaptoethanol, 200 µM each dATP, dCTP (a mix of unlabelled and [ <sup>32</sup> P]-dCTP), dGTP, dTTP, 12.5 µg activated calf thymus DNA, in a total volume of 50 µl.

**Protocol**

The following basic protocol serves as a general guideline for PCR amplification. Optimal reaction conditions (incubation times and temperatures, primers, MgCl<sub>2</sub>, and template DNA) vary and need to be optimized.

1. Thaw vial, mix and place on ice.
2. Remove 25 µl and add primers, template and extra MgCl<sub>2</sub> (if required) in volume of 25 µl to bring the total reaction volume to 50 µl.

Components	Volume	Final Concentration
2X PCR Master Mix	25 µl	1X
Primer	Variable	0.1–0.5 µM
Template DNA	≥1 µl (as required)	-
Autoclaved, distilled water	to 50 µl	-

2. Cap the tubes and centrifuge briefly to collect the contents.
3. Incubate tubes in a thermal cycler at 94°C for 9-12 min to completely denature the template.
4. Perform 25-35 cycles of PCR amplification as follows:

Denature	0.5–1 min	94°C
Anneal	0.5–1 min (depends on T <sub>m</sub> of primers)	50–68°C
Extend	1 min per kb	72°C
Final Extend	10 min	72°C

5. If reactions are not to be used immediately, keep them on ice for a maximum of 3 hours. Long term storage of the master mix should be at -20°C.